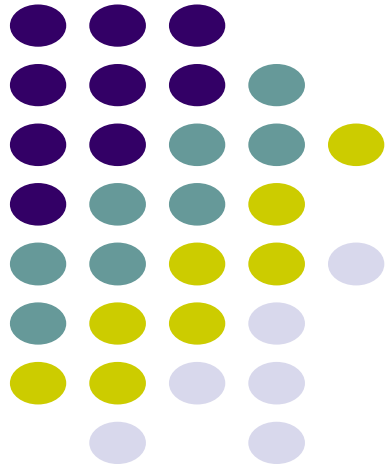
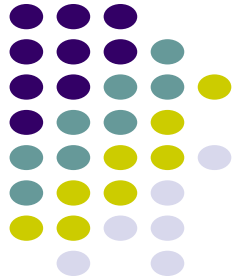
Diagnosis of seed borne pathogens



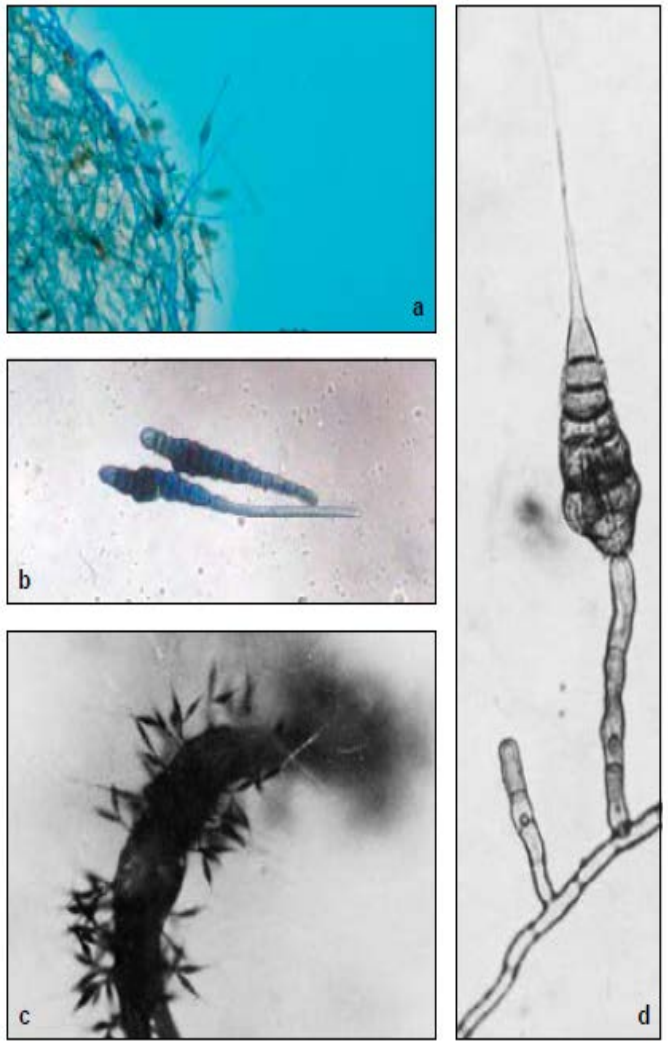
Seed Health Testing Methods



- Blotter method
- Agar method (Malt, Potato Dextrose, Yeast Dextrose chalk, etc.)
- Paper towel method
- Embryo Extraction method
- De-hulling and embryo extraction
- Extraction and Agar plating method
- Extraction and Polymerase Chain Reaction method
- ELISA method

Blotter method

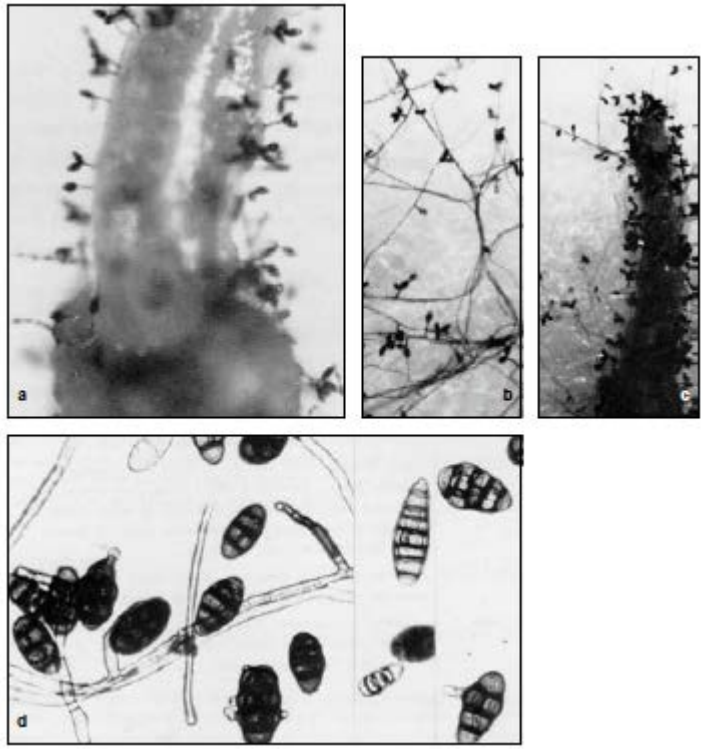
- ***Alternaria dauci*** **in** ***Daucus carota*** **(carrot) seed**



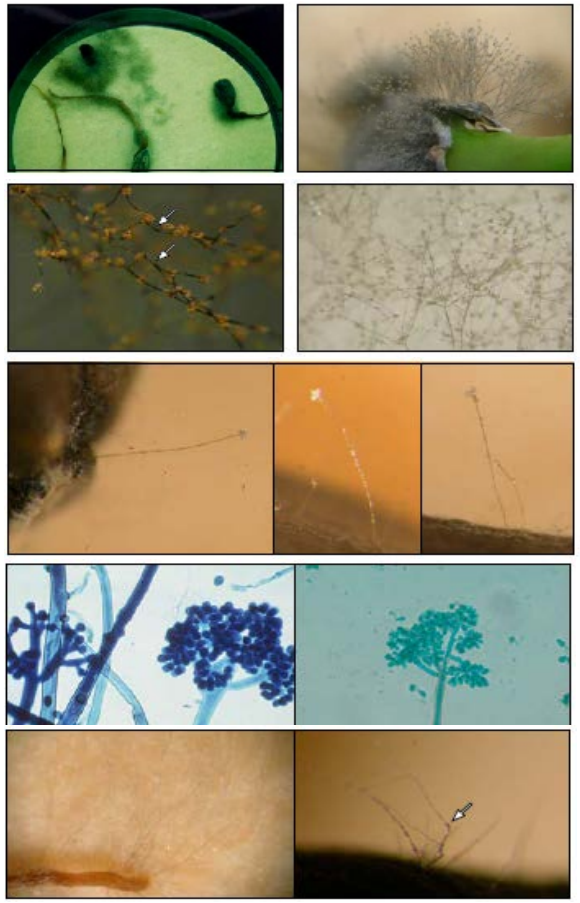
Blotter Methods

- Place three 90 mm filter papers in each plate and soak with sterile distilled/deionised water. Drain away excess water.
- Plating: Aseptically place 10 seeds, evenly spaced, on the surface of the filter paper in each plate.
- Incubate for 3 d at 20 ±2 °C in the dark.
- Transfer plates to freezer and maintain at –20 ±2 °C for 24 h.
- After freezing, incubate for 6 d at 20 ±2 °C with alternating 12 h periods of darkness and NUV light. Plates should be approx. 25 cm below the lights and should not be stacked.
- Examine seeds under a stereoscopic microscope at ×30 for fungal growth and up to ×80 magnification for identification of conidia. Conidiophores are simple or slightly branched, arising singly or in small groups from the surface of the seed or on aerial mycelium. Conidia are usually solitary, obclavate, up to 450 µm long (including beak), pale olivaceous brown at first, becoming brown with age, with a long pale beak up to 3 times the length of the body. Groups of sunken conidia are sometimes visible by the emerging clusters of their bright long beaks. Compare with positive control. Record the number of infected seeds in each plate.

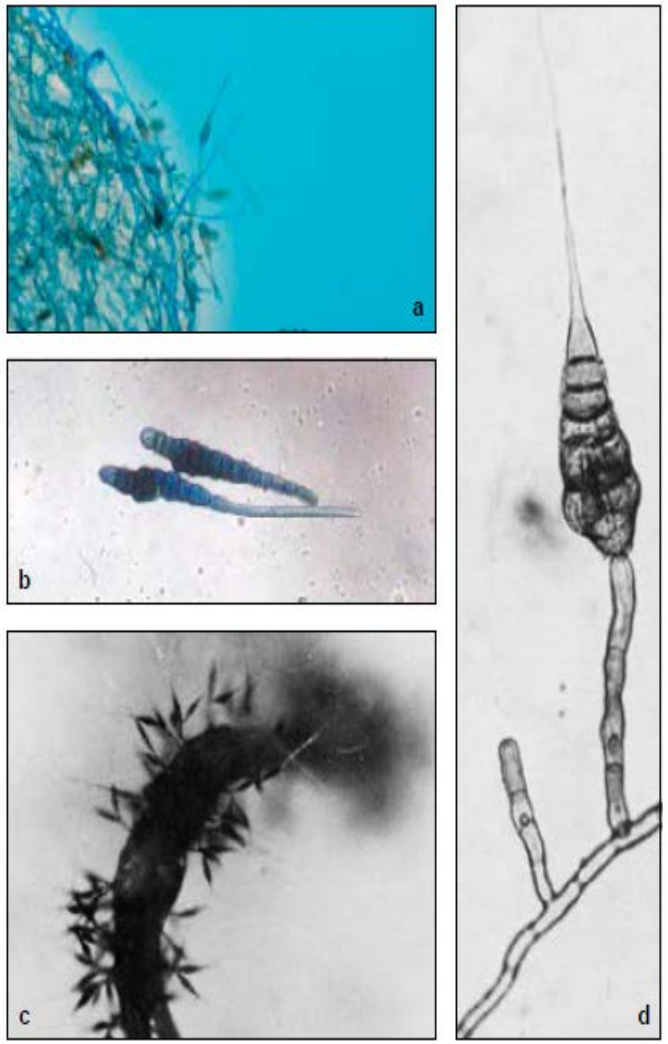
Alternaria radicina in *Daucus carota* (carrot) seed



Botrytis cinerea in *Helianthus annuus* (sunflower) seed

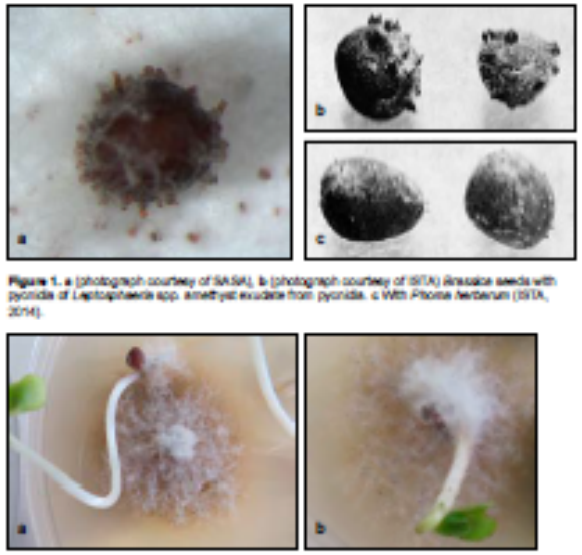


- **Detection of *Alternaria radicina* in *Daucus carota* (carrot) seed by malt agar method**

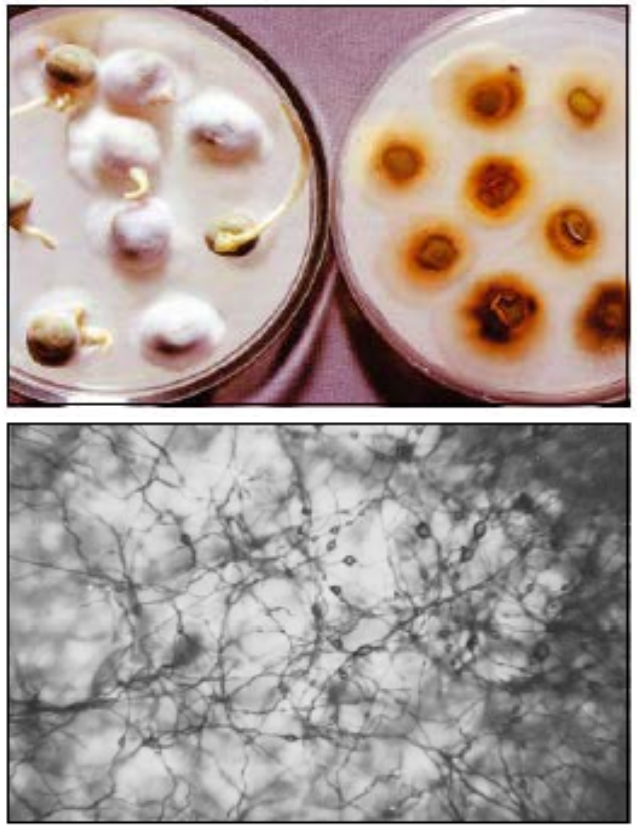


Agar method

Leptosphaeria maculans **and** ***Plenodomus biglobosus*** **in** ***Brassica*** **spp. seed**



Ascochyta pisi **in** ***Pisum sativum*** **(pea) seed**

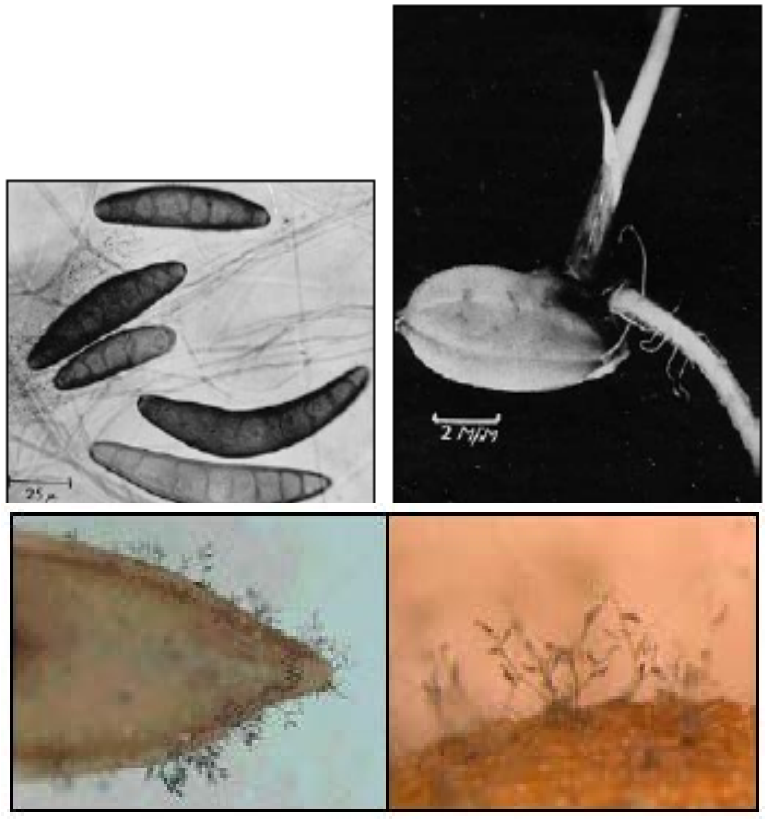


Methods

- Plating: Aseptically place a max. of 10 seeds, evenly spaced, on the agar surface of each malt agar plate.
- Incubate plates for 10 d at 20 ±2 °C, with alternating 12 h periods of darkness and NUV light. Plates should be approx. 25 cm below the lights and should not be stacked.
- Subculture a reference culture to a malt agar plate at the same time the seeds are plated and incubate with the test plates.
- Examine plates visually, and under a stereoscopic microscope at ×30 magnification, for fungal growth. Use a magnification of ×50 – ×80 for identification of conidia. Colonies of *Alternaria radicina* are irregular to circular with luxurious aerial mycelium, dark olive grey to greyish-black from above, bluish-black from below. Conidiophores are simple or occasionally branched, arising usually singly from the surface of the seed, on the emerging radicle or on aerial mycelium. Conidia are produced singly or in chains of 2, or rarely 3, ellipsoidal or barrel shaped, with little evidence of beak, up to 75 µm long, olivaceous brown. Under the stereoscopic microscope, conidia appear blackish and glossy. Compare with positive control. Record the number of infected seeds in each plate (CCP).

***Colletotrichum lindemuthianum* in *Phaseolus vulgaris* (bean) seed**

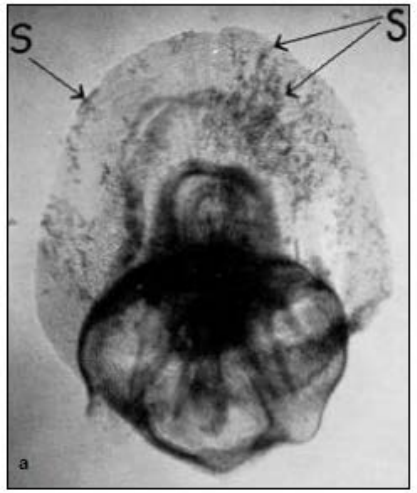
***Bipolaris oryzae* in *Oryza sativa* (rice) seed**

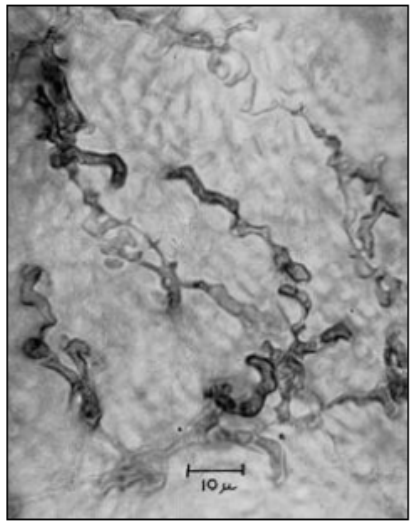


Rolled paper towel method

Embryo Extraction method

- **Detection of *Ustilago nuda* in *Hordeum vulgare* (barley) seed by embryo extraction**





- Working sample of embryo method
  - Two replicates of 100–120 g containing, depending on 1000-seed weight, 2000–4000 seeds.
- Extraction and clearing of embryos
  - Place the seeds in 1 L of a freshly prepared 5 % aqueous solution of sodium hydroxide (NaOH) and maintain at 20 °C for 24 h.
  - After soaking, the entire sample should be transferred to a suitable container and washed in warm water to separate the embryos, which appear through the softened pericarps.
  - Collect the embryos in a sieve of 1 mm mesh. Additional sieves of larger mesh can be used to collect pieces of endosperm and chaff.
  - Transfer the embryos to a mixture of equal quantities of glycerol and water in which further separation of the embryos and chaff can be made.
  - Transfer the embryos to a beaker containing 50 mL of lactic acid solution and clear them by maintaining the lactic acid solution at boiling point for approximately 5 min. in a fume cupboard.
  - Transfer the embryos to fresh glycerol for examination. The scutellum becomes more transparent when embryos are left in glycerol for 1–2 h, making examination easier.

- Examination
  - Examine embryos at ×16–25 magnification with adequate substage illumination for the characteristic golden brown mycelium of *U. nuda.*
  - Mycelium is approximately 3 µm thick, is golden brown in colour and visible without a stain. Infection may vary from a few strands of short hyphae to complete invasion of the scutellum tissues. Occasionally fungi other than *U. nuda* occur in the scutellum but are usually darker in colour and quite distinct. When cell walls become discoloured they may be confused with mycelium of U. nuda, but this can be checked by examination at ×50 or higher magnification. Compare with positive control (reference material).

- **Detection of *Ustilago nuda* in *Hordeum vulgare* (barley) seed by dehulling and embryo extraction**

- **Methods**
- **Dehulling**
- Place the working sample in a glass beaker with 25– 37 % H_2SO_4 until the seeds are covered.
- Incubate in an oven at 75 °C for 50 min. or until the seeds turn a medium-brown colour.
- Carefully pour off the H_2SO_4 solution. Rinse seeds by pouring water into the beaker, gently mix and pour out the water. Add new water and remove the loosened hulls by stirring robustly with a rod. Remove hulls by carefully removing the water. If hulls remain, add new water, and either use an electric hand mixer at low speed (maximum 3 min.) or continue stirring. Repeat procedure until all hulls are removed. Be careful not to lose any kernels (seed without hulls).
- **Embryo extraction :** As mentioned earlier

Extraction and Polymerase Chain Reaction method

- **Detection of *Xanthomonas campestris* pv. *campestris* in *Brassica* spp. seed**

ELISA method

- Detection of *squash mosaic virus, cucumber green mottle mosaic virus* and *melon necrotic spot virus* in cucurbit seed

Grow-out test

- *Squash mosaic virus*